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THE INHIBITORY INNERVATION OF THE TAENIA OF THE GUINEA-PIG CAECUM

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SUMMARY

1. The inhibitory innervation of the taenia of the guinea-pig caecum has been studied, after blocking the responses to stimulation of excitatory cholinergic nerves with atropine.

2. Stimulation of the perivascular nerves supplying the taenia caused relaxations. These nerves had properties which were typical of sympathetic post-ganglionic adrenergic nerves. The relaxations caused by stimulation were maximal at frequencies of stimulation above 30 pulses/sec and they were abolished by bretylium, guanethidine and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP).

3. The taenia is also innervated by intramural inhibitory nerves with their cell bodies in Auerbach's plexus. These nerves can be excited by electrical stimulation of the taenia or by the application of ganglion-stimulating drugs.

4. The intramural inhibitory nerves have different properties from sympathetic adrenergic nerves. Relaxations in response to stimulation were maximal with frequencies of stimulation of about 5 pulses/sec and they were not blocked by bretylium, guanethidine or DMPP.

5. Preganglionic cholinergic fibres in the caecal wall make synaptic connexions with the intramural inhibitory neurones.

6. The role of the intramural inhibitory neurones in intestinal activity and their possible connexions with the central nervous system have been discussed.

INTRODUCTION

Many studies have been made of the electrophysiology of taeniae from the guinea-pig caecum (Bülbring, 1955; Burnstock, 1958*a*, *b*; Holman 1958; Bülbring & Kuriyama, 1963*a*, *b*, *c*; Kuriyama, 1963). The concentrations and fluxes of various ions in this tissue under a variety of conditions are also known (Born & Bülbring, 1956; Goodford, 1962, 1964; Nagasawa, 1963, 1964). In spite of this extensive use of the taenia, little is known about its innervation and its responses to nerve stimulation.

This paper describes the mechanical responses of the isolated taenia produced by stimulation of sympathetic perivascular nerves and compares them to those produced by stimulation of nerves within the taenia itself and of other nerves in the wall of the caecum. Particular attention has been paid to the responses to stimulation of inhibitory nerves, since one purpose of these experiments was to provide a basis for the study of the electrophysiology of inhibitory transmission in smooth muscle (Bennett, Burnstock & Holman 1966*a*, *b*). The effect of some drugs have also been investigated: in particular catecholamines and ganglion stimulants, and drugs which produce blockade of sympathetic transmission, nerve conduction or ganglionic stimulation.

Preliminary reports of some aspects of this work have been published (Burnstock, Campbell, Bennett & Holman, 1963, 1964).

METHODS

Guinea-pigs of either sex, weighing between 200 and 500 g, were used. The animals were stunned and bled to death, and the taeniae were dissected from the caecum. This muscle has previously been referred to as the taenia coli, but the name 'taenia caeci' would be more appropriate. The longitudinal muscle of the guinea-pig colon is not arranged in taeniae.

Four types of isolated preparation of the taenia have been used.

Taenia strip preparation. Strips of either of the two exposed taeniae were cut away from the surface of the caecum with fine scissors. The cut was made as shown in Fig. 1*a* and the preparation contained the circular muscle underlying the taenia, but not the mucosa or submucosa. These strips were then cut into lengths of about 2 cm. For stimulation of nerves within the taenia, the strip was passed through two loops of platinum, separated by 3 mm which allowed free movement of the preparation.

Superficial strips of taenia. In some experiments a surface strip of the taenia was cut which contained only longitudinal muscle as shown in Fig. 1*b*. Very large guinea-pigs (800 g) were used for this preparation. The intention in cutting surface strips was to exclude the cell bodies of the neurones in Auerbach's plexus, which lie between the longitudinal and circular muscle layers.

Perivascular nerve-taenia preparation. The caecum was arranged so that the ileo-caecal junction was exposed. The large blood vessel lying between the terminal ileum and the caecum was cut free from the mesentery. Lateral branches of the large blood vessel were severed with the exception of one, or sometimes two, blood vessels extending to the caecum at a point about 2 cm from the ileo-caecal junction. The blood vessel branches which were preserved usually ran adjacent to a large lymph node lying in the angle between the caecum and the ileum. The large blood vessel was divided as far away as possible from the caecum. The blood vessel branches passed on to the caecum and ran over the caecal wall to the taenia, where they branched into arcades. Cuts were then made through the caecal wall on either side of these branches at about 5 mm distance from them; these cuts were extended to the taenia. The taenia and the underlying caecal wall were then removed, together with the blood vessels. The taenia was cut so that less than 1 cm was left on the proximal side of the blood vessel, while 2 or 3 cm were left on the distal side. This preparation is illustrated diagrammatically in Fig. 1*c*. The mesenteric blood vessels were either tied over two platinum wires or passed through two platinum loops 3 mm apart. These electrodes were at least 1 cm away from the wall of the caecum. In some experiments an additional pair of electrode rings were placed around the proximal end of the strip of taenia.

Caecal wall preparation. The wall of the caecum lying between two taeniae was cut in the circular direction to form a flap, one end of which was left attached to a taenia. A length of about 3 cm of taenia was cut free, with the flap of caecal wall attached to one side. A diagram illustrating this procedure is shown in Fig. 1*c*. The free end of the flap was attached to a pair of platinum electrodes. The flap was chosen from a portion of the wall that was free from blood vessels (visible to the naked eye), in order to avoid, as far as possible, stimulation of perivascular nerves.

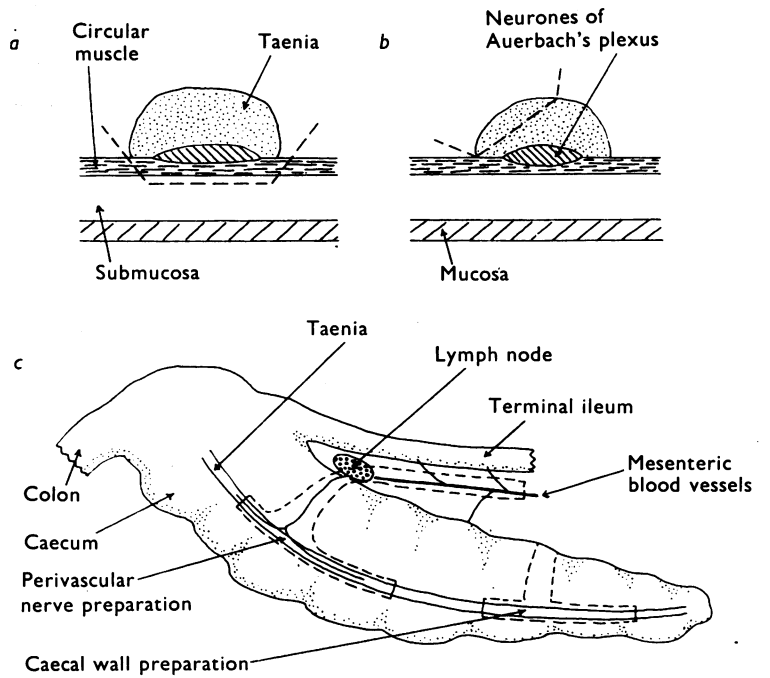

Fig. 1. Preparations of the guinea-pig taenia. (*a*) Taenia strip preparation. (*b*) Ganglion-free taenia strip preparation. (*c*) Perivascular nerve-taenia and caecal wall preparations. *a* and *b* are diagrammatic transverse sections of the caecal wall, *c* is a diagram of the caecum. The interrupted lines indicate where the preparations are cut.

The preparations were suspended in an organ bath containing 40 ml. of modified Krebs's solution (Bülbring, 1953 or McEwen, 1956) bubbled with 95% O_2 and 5% CO_2. The temperature was maintained at between 35 and 37° C with a heated water-bath. The free end of the preparation was tied to an isotonic frontal-writing lever, which exerted a load of about 1 g and recorded with approximately sixfold magnification on a smoked drum.

An electronic stimulator was used to deliver square-wave pulses at different frequencies. The pulse duration was usually 1 msec. Strengths of stimulation causing near-maximal responses at any particular frequency of stimulation were used. Trains of pulses, lasting for 10 sec, were given at intervals of not less than 4 min.

Drugs used were: Acetylcholine chloride, adrenaline bitartrate, atropine sulphate, bretylium tosylate, cinchocaine hydrochloride, 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), dopamine hydrochloride, guanethidine sulphate, hexamethonium bromide, 5s-hydroxytryptamine creatinine sulphate (5-HT), hyoscine hydrobromide, nicotine bitartrate, noradrenaline bitartrate, pentolinium tartrate, and procaine hydrochloride. All concentrations are expressed in terms of the above salts.

RESULTS

The tone and the spontaneous activity of the taeniae varied considerably from one preparation to another. Tone was estimated from the extent of the relaxation produced by catecholamines. There was a relation between the tone and type of spontaneous activity.

From observations on 132 preparations the following patterns could be distinguished: (*a*) When the tone was low the spontaneous beats occurred at a rate of about 10/min; noradrenaline inhibited the beats but did not

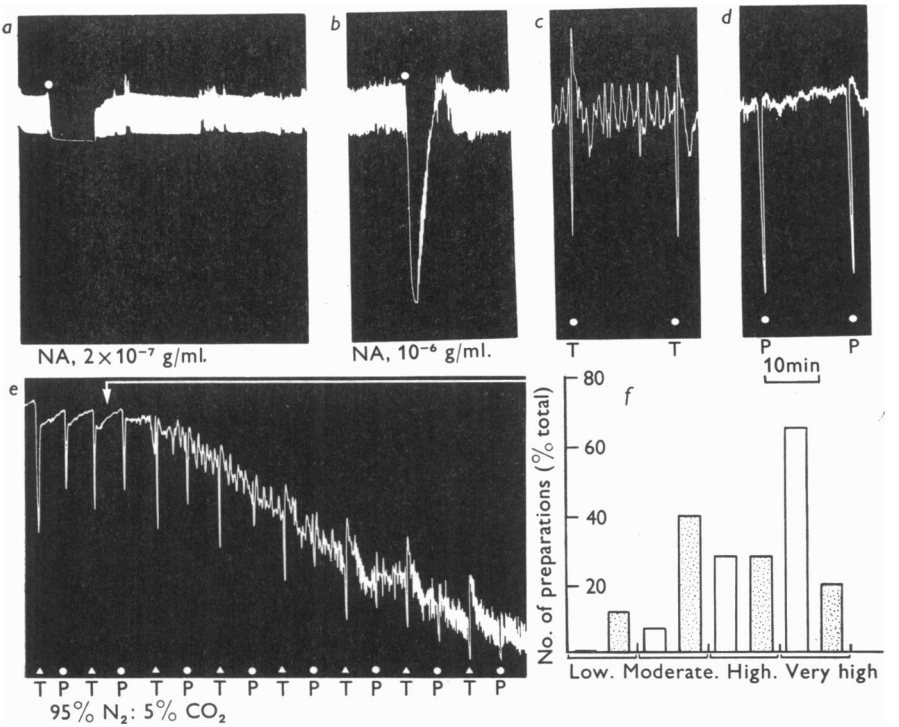

Fig. 2. Tone and rhythmicity of atropinized preparations of the taenia. (*a*) Low tone. Taenia strip preparation. Noradrenaline (NA, 2×10^{-7} g/ml., applied for 8 min) was added at the dot. (*b*) Moderate tone. Taenia strip preparation. Noradrenaline (NA, 10^{-6} g/ml., applied for 1 min) was added at the dot. (*c*) High tone. Taenia strip preparation. The taenia was stimulated with 20 pulses/sec (T, at dots). (*d*) Very high tone. Perivascular nerve-taenia preparation. The perivascular nerves were stimulated with 30 pulses/sec (P, at dots). (*e*) Change in rhythmicity during a fall of tone. Perivascular nerve-taenia preparation. The tone was initially very high and fell, during bubbling with 95% N_2 and 5% CO_2. The perivascular nerves (P, at dots) and the taenia (T, at triangles) were stimulated with 20 pulses/sec. (*f*) Histogram, to show the percentage distribution of tone amongst 82 perivascular nerve-taenia preparations (open columns) and 50 taenia strip preparations (dotted columns). Time marker for (*a*)–(*e*), 10 min.

cause appreciable relaxation (Fig. 2*a*). (*b*) Preparations with a moderate tone, in which noradrenaline caused relaxation, had a slightly slower and less regular beat (Fig. 2*b*). (*c*) High tone preparations had irregular beats at a mean rate of about one per minute (Fig. 2*c*). (*d*) In very high tone preparations the amplitude of beating was small (Fig. 2*d*). Although these categories of tone are arbitrary, they were useful in assessing the responses to inhibitory and excitatory stimuli, since the extent of the relaxation produced by an inhibitory stimulus was greater the higher the tone and the extent of the contraction caused by an excitatory stimulus was less. Figure 2*e* shows an example of the effect of a reduction of the tone on the responses to electrical stimulation. This figure also shows that the pattern of spontaneous activity changed in accord with the change in tone. With experience, observations of spontaneous activity can serve as a guide to the tone of a preparation. The tone was generally higher when the whole thickness of the underlying caecal wall was attached to the taenia (as in perivascular nerve preparations) than when the taenia alone was used (Fig. 2*f*). This difference was possibly due to the difference in the degree of stretching, since the weighting on the lever was the same in either case.

Responses to electrical stimulation

Perivascular nerve-taenia preparation

Stimulation of the perivascular nerves at frequencies of 3 pulses/sec or more caused relaxations of the taenia. There was usually no response to stimulation at frequencies between 1 and 3 pulses/sec, but in a few preparations there was a small relaxation or a small contraction.

Responses after atropine. The contractions caused by acetylcholine (10^{-8} to 10^{-7} g/ml.) were abolished by atropine or hyoscine (10^{-8} g/ml.). The relaxations produced by perivascular nerve stimulation were enhanced after atropine or hyoscine (10^{-8} to 10^{-7} g/ml.). These observations suggest the presence of cholinergic fibres in the perivascular nerve supply. A single pulse was usually ineffective, but stimulation at 1 pulse/sec caused relaxation in most of the preparations and stimulation at 2 pulses/sec always caused relaxation. The amplitude of the relaxation increased as the frequency of stimulation was increased (Fig. 3*a*). The lowest frequency of stimulation which caused maximal responses was between 30 and 70 pulses/sec. Maximal relaxations were produced by stimulation with pulse widths of 1·2–1·5 msec.

The relaxations of the taenia caused by stimulation of the perivascular nerves appeared after a latent period of about 1 sec (Fig. 4*a*). Sometimes a momentary increase in the rate of rise of a spontaneous beat occurred at the beginning of stimulation. When stimulation was stopped, relaxation

continued for a period before the muscle started to recover (Fig. 4*b*). The duration of this period increased as the frequency of stimulation was increased, becoming as long as 20 sec after stimulation with 50 pulses/sec (Fig. 4*e*). In many experiments the relaxation was followed by an after-contraction which persisted for up to 1 min (Fig. 4*b*).

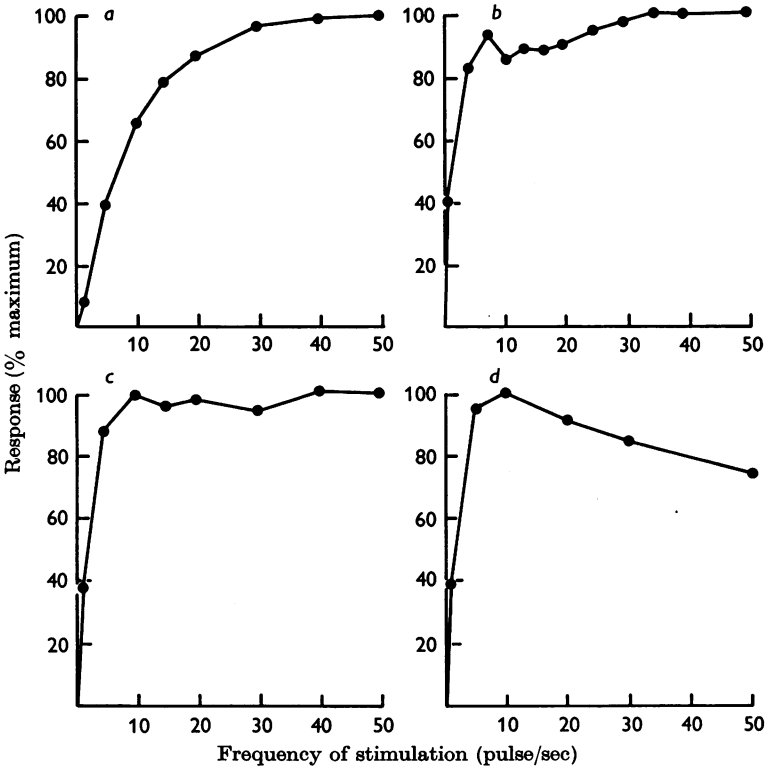

Fig. 3. Frequency–response curves for stimulation of the taenia or of the perivascular nerves in the presence of atropine. (*a*) Perivascular nerve stimulation. The curve approached a maximum at 50 pulses/sec. (*b*) Taenia strip preparation. The curve showed a peak at 8 pulses/sec and rose to a second maximum at 35 pulses/sec. (*c*) Taenia strip preparation. The responses to stimulation became maximal at 10 pulses/sec and remained fairly uniform at higher frequencies. (*d*) Taenia strip preparation. The relaxations reached a peak at about 10 pulses/sec and declined with higher frequencies.

Guanethidine (10^{-6} g/ml.) or bretylium (5×10^{-6} g/ml.) abolished the responses to stimulation of the perivascular nerves at all frequencies (Fig. 5*a*, *c*). The rate of development of the blockade caused by guanethidine was greater at the lower frequencies of stimulation. Thus the time taken to reduce the responses by a half was 7–10 min at 10 pulses/sec,

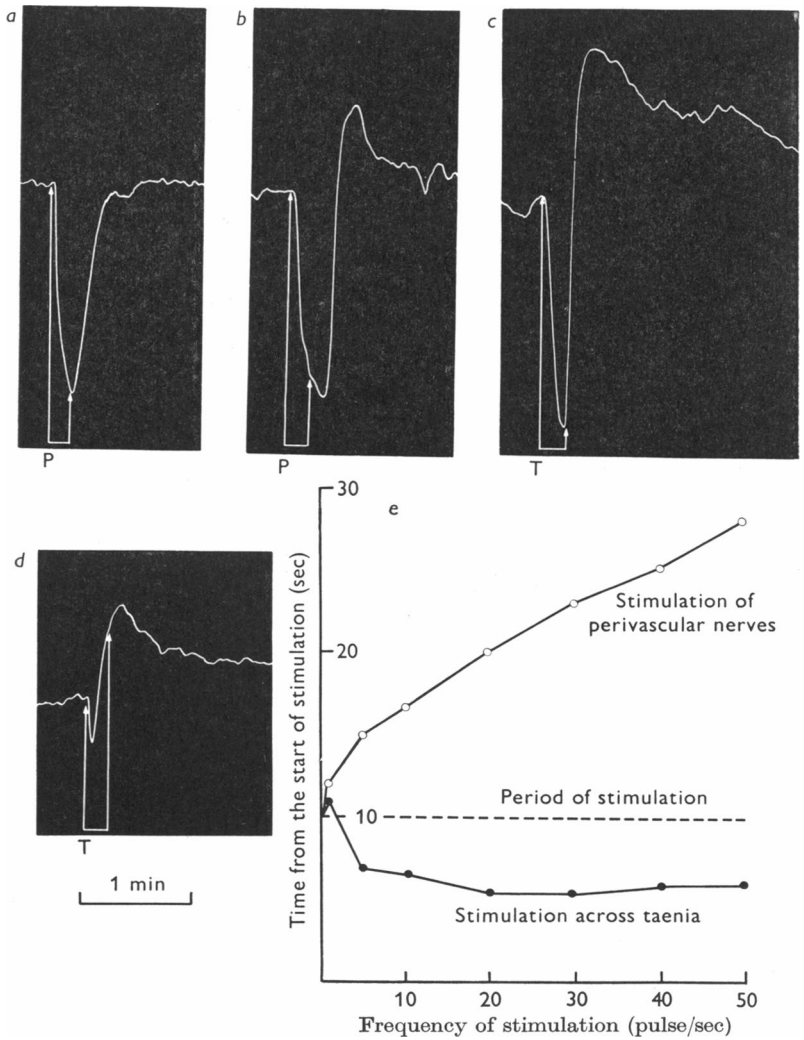

Fig. 4. Responses to stimulation of the taenia and of the perivascular nerves after atropine. (*a*) Perivascular nerve-taenia preparation. The perivascular nerves were stimulated with 10 pulses/sec (P, between the arrows). (*b*) Perivascular nerve-taenia preparation. The perivascular nerves were stimulated with 30 pulses/sec (P, between the arrows). Note the delay in the start of recovery compared with (*a*). (*c*) Stimulation of the taenia in the same preparation as in *b*, with 30 pulses/sec (T, between the arrows). Note the larger after-contraction compared with (*b*). (*d*) Perivascular nerve-taenia preparation. Stimulation of the taenia with 30 pulses/sec (T, between the arrows). Note that the preparation starts to contract during stimulation. (*e*) Perivascular nerve-taenia preparation. The graph shows the relationship between the frequency of stimulation and the time, from the start of stimulation, at which the preparation first started to recover its tone. Stimulation of the perivascular nerves (○) or of the taenia (●). For explanation, see text. Time marker for (*a*)–(*d*) 1 min.

but was 13–16 min at 40 pulses/sec. Responses did not return within 1 hr of washing either drug from the bath.

The ganglion-blocking drugs pentolinium (10^{-5} g/ml.) and hexamethonium (10^{-4} g/ml.) did not reduce relaxations caused by stimulation of the perivascular nerves. In fact, these drugs sometimes caused a slight increase in the amplitude of the responses.

These findings demonstrate that relaxations of the taenia produced by perivascular nerve stimulation are due to post-ganglionic sympathetic fibres.

Taenia strip preparation

Electrical stimulation applied to one end of the taenia strip at frequencies of 1–50 pulses/sec caused relaxation in two thirds of the preparations. In the remaining experiments, stimulation caused contraction over at least some part of this range of frequencies.

Responses after atropine. The contractions produced by electrical stimulation of the taenia were reversed to relaxations after atropine (10^{-7} g/ml.), as shown in Fig. 6*a*, or after hyoscine.

Stimulation of the taenia with a single pulse always caused relaxation. The amplitude of the relaxation increased with increasing frequencies of stimulation until a peak was reached at about 5 pulses/sec (Fig. 3*b*, *d*), although sometimes the peak occurred at as low as 1 pulse/sec. As the frequency was raised, the amplitude of the responses either increased further until it became maximal at 30 pulses/sec or more (Fig. 3*b*), or remained fairly constant (Fig. 3*c*), or continually decreased (Fig. 3*d*). The pulse duration giving maximal relaxations to stimulation of the taenia at 10–20 pulses/sec was about 0·8 msec.

The responses to stimulation of the taenia differed in some other respects from those obtained when the extrinsic perivascular nerves were stimulated (Fig. 4*c–e*). The latency was rather shorter, being about 0·8 sec. The rates of relaxation and of contraction during recovery were generally greater than in relaxations of comparable magnitude caused by stimulation of the perivascular nerves. When the taenia was stimulated at frequencies of less than about 5 pulses/sec, it usually continued to relax throughout the 10 sec period of stimulation. At higher frequencies of stimulation, the taenia often started to return to its former length while stimulation was continuing (Fig. 4*c–e*) and sometimes returned to or even contracted beyond the resting length within the 10 sec burst of stimulation at 30 pulses/sec (Fig. 4*d*). There was a marked after-contraction in most preparations and it was always greater than that following relaxations of comparable magnitude caused by stimulation of the perivascular nerves (Fig. 2*c* and *d* and the last few responses in Fig. 2*e*; Fig. 4; Fig. 5*c*). In

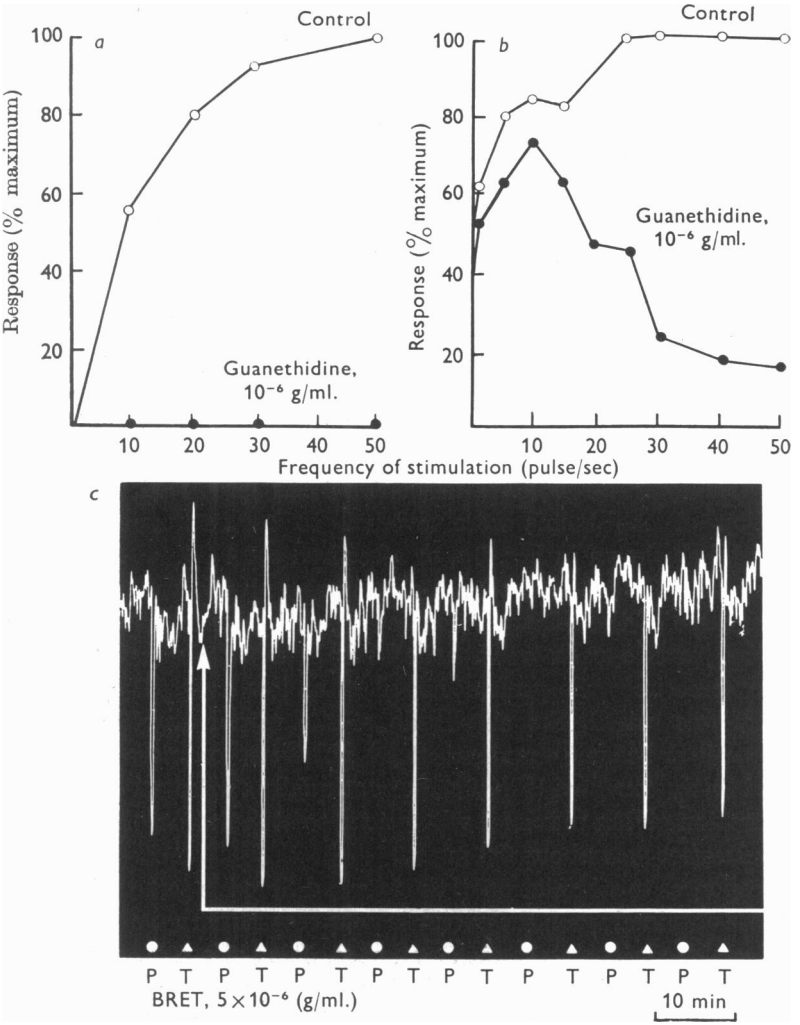

Fig. 5. Effects of adrenergic neurone-blocking drugs on responses to stimulation of the taenia or of the perivascular nerves after atropine. (*a*) Frequency–response curves for stimulation of the perivascular nerves before (○) and after (●) the addition of guanethidine (10^{-6} g/ml.). Note the complete blockade of the responses by guanethidine. (*b*) Taenia strip preparation. Frequency–response curves obtained by stimulation before (○) and after (●) the addition of guanethidine (10^{-6} g/ml.). Note that the peak at 10 pulses/sec is not abolished by guanethidine but that the maximum at higher frequencies disappears. (*c*) Perivascular nerve-taenia preparation. Bretylium (BRET, 5×10^{-6} g/ml.) abolished responses to stimulation of the perivascular nerves at 30 pulses/sec (P, at dots), but only reduced responses to stimulation of the taenia with 10 pulses/sec (T, at triangles). Time marker, 10 min.

some preparations, especially those with low tone, the after-contraction persisted for up to 10 min before recovery was complete.

The ganglion-blocking drugs pentolinium (10^{-5} g/ml.) and hexamethonium (10^{-4} g/ml.) did not reduce the relaxations caused by stimulation of the taenia.

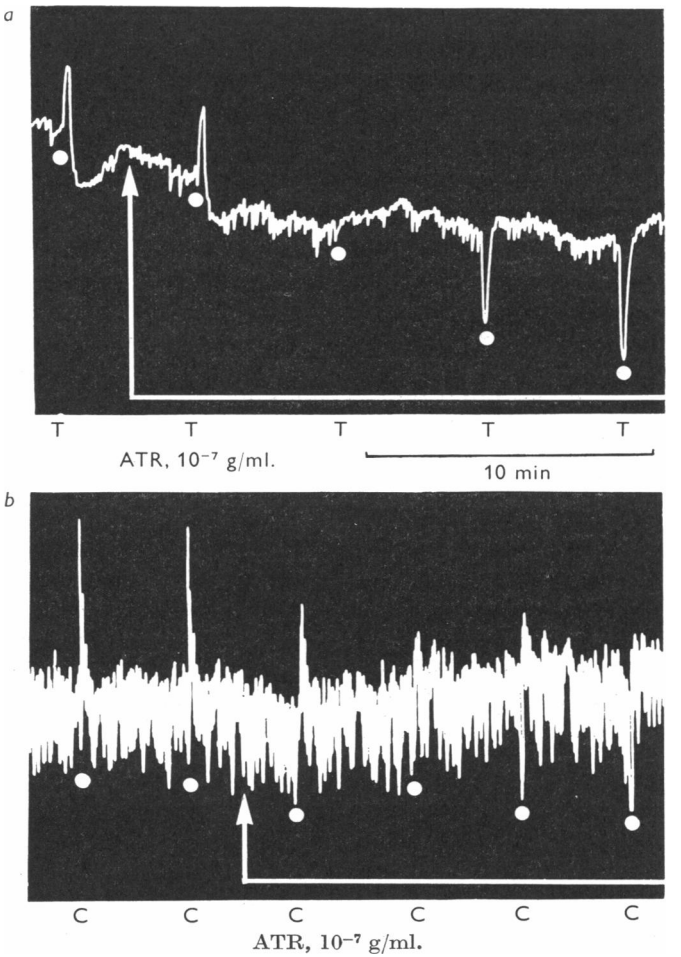

Fig. 6. Effect of atropine on responses to electrical stimulation. (*a*) Taenia strip preparation. Atropine (ATR, 10^{-7} g/ml.) abolished the contractions caused by stimulation of the strip with 50 pulses/sec (T, at dots), and revealed relaxations. (*b*) Caecal wall preparation. Atropine (ATR, 10^{-7} g/ml.) abolished the contractions caused by stimulation of the flap of caecal wall with 5 pulses/sec (C, at dots) and revealed relaxations. Time marker, 10 min. applies to both *a* and *b*.

Guanethidine (10^{-6} g/ml.) or bretylium (5×10^{-6} g/ml.) caused very little reduction of relaxations produced by stimulation of the taenia at less

than about 10 pulses/sec (Fig. 5*b*, *c*). The effect of these drugs on responses to stimulation at higher frequencies depended on the initial shape of the frequency–response curve. When the curve had the shape shown in Fig. 3*d*, these drugs caused only a slight reduction of all responses. However, when the curve had either of the shapes shown in Fig. 3*b* or *c*, with maximal responses occurring at about 30–50 pulses/sec, guanethidine and bretylium caused a considerable reduction in the amplitude of the responses. In general, the effect of guanethidine and bretylium on the frequency–response curve was to reduce responses to stimulation with high frequencies more than those to stimulation with low frequencies (Fig. 5*b*). Relaxations, which were maximal at about 5 pulses/sec, always remained, even when the concentrations of the drugs was increased up to 100-fold. These results suggested that the relaxations caused by stimulation of the taenia strip in the presence of guanethidine or bretylium were not entirely due to stimulation of the extensions of the perivascular nerves within the preparation.

Local anaesthetic drugs. Cinchocaine and procaine were used for the purpose of blocking nerve conduction in order to test whether the relaxations caused by stimulation of the taenia strip were due to excitation of nerve fibres within the tissue.

Cinchocaine (5×10^{-5} g/ml.) decreased the tone of the taenia, but it abolished the responses to stimulation of the taenia and of the perivascular nerves before the reduction in tone was developed. Procaine, in concentrations greater than 10^{-5} g/ml., caused a rise in tone. Responses to perivascular nerve stimulation were almost abolished by a concentration of 2×10^{-4} g/ml., as shown in Fig. 7*a*. Higher concentrations of procaine were needed to abolish the response to stimulation of the taenia. Figure 7*b* shows the relation between the concentration of procaine and the percentage reduction in the responses to each type of stimulation. Each of the calculated regression lines were highly significant (P, 0·001, analysis of variance) and the two lines did not differ significantly from the parallel (t test, P, 0·9).

These findings suggest that the relaxations produced by stimulation of the taenia are due to excitation of nerves. The fact that a higher concentration of procaine was required to block these nerves may be explained by their having a larger diameter than the perivascular nerves, and this is compatible with the finding that the maximal relaxation was obtained with a shorter pulse. However, the relaxations produced by noradrenaline (2×10^{-7} g/ml.) were reduced by procaine (3×10^{-4} g/ml.), as shown in Fig. 7*c*, and a higher concentration of procaine sometimes abolished them. Bucknell & Whitney (1964) showed that procaine reduced the responses of the human taenia coli caused by adrenaline. Cinchocaine (5×10^{-5} g/ml.) also reduced the responses of the guinea-pig

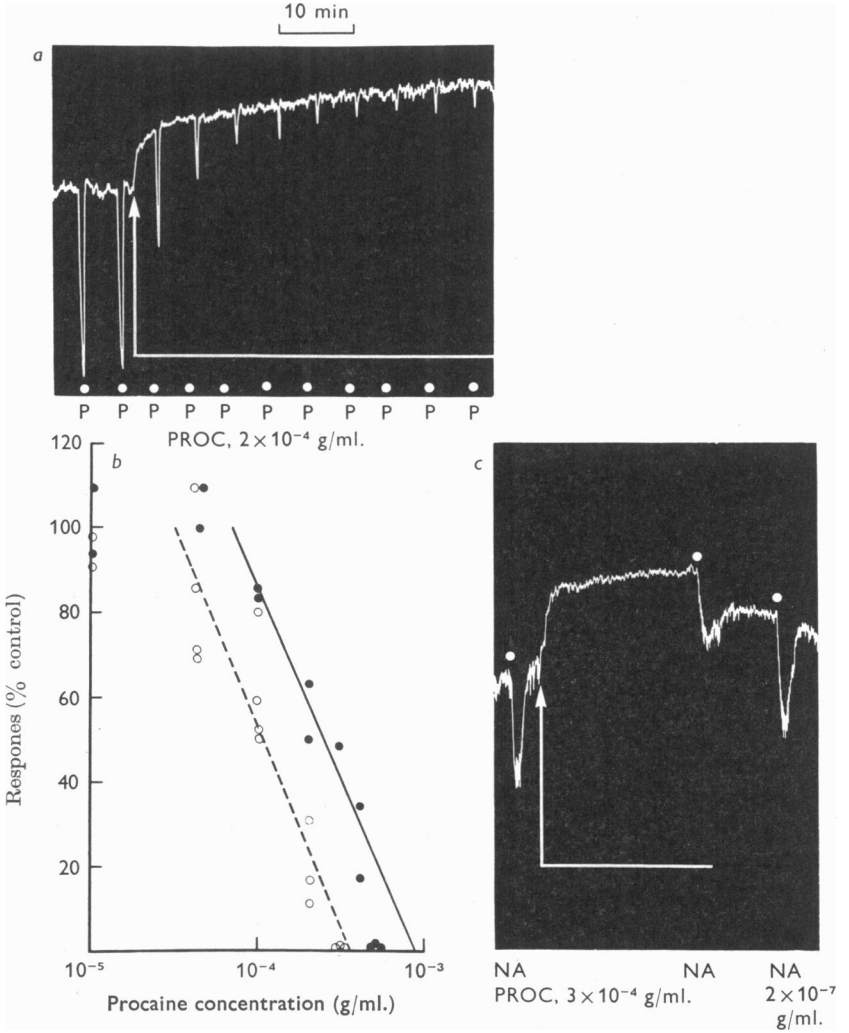

Fig. 7. (*a*) Effects of procaine on the atropinized taenia. The addition of procaine (PROC, 2×10^{-4} g/ml.) caused a sustained rise in tone and a diminution of responses to stimulation of the perivascular nerves with 30 pulses/sec (P, at dots). (*b*) Log dose–effect curves for the blockade by procaine of responses to stimulation, at 30 pulses/sec, of the perivascular nerve (○, interrupted line) and of the taenia (●, full line) in perivascular nerve-taenia preparations. Ordinate: amplitude of relaxations as a percentage of those obtained before procaine. Abscissa: concentration of procaine, log scale. The lines are the calculated regression lines for responses reduced to between 90 and 10 %. (*c*) Taenia strip preparation. Procaine (PROC, 3×10^{-4} g/ml.) reduced responses to noradrenaline (NA, 2×10^{-7} g/ml., at dots). Time marker for (*a*) and (*c*) 10 min.

taenia to noradrenaline. These findings detract from the value of the observations on responses to electrical stimulation, since it follows that at least part of the reduction in the response produced by the local anaesthetics may have resulted from diminished responsiveness of the smooth muscle.

Caecal wall preparation

Electrical stimulation of a flap of caecal wall which was left attached to the taenia caused contraction in two thirds of the preparations and relaxation in the remainder. After atropine, the contractions were abolished and relaxations appeared, as shown in Fig. 6*b*. These relaxations resembled those produced by stimulation applied to the taenia itself in that guanethidine or bretylium caused a considerable reduction in the responses to stimulation at above 20 pulses/sec but had less effect on responses to lower frequencies of stimulation: the relaxations were never abolished. However, the ganglion-blocking drug pentolinium (10^{-5} g/ml.) reduced the relaxations caused by stimulation of the flap of caecal wall by between 30 and 60 % (Fig. 8). This effect of pentolinium was also seen on preparations which had previously been treated with guanethidine. Pentolinium caused similar reductions of the relaxations whether the flap was attached to the proximal or the distal end of the segment of taenia. This finding suggests the presence in the caecal wall of nerve fibres which make synaptic connexions with inhibitory neurones.

Effects of ganglion stimulating drugs (nicotine and DMPP). Further evidence for the presence of inhibitory neurones in the taenia strip preparation was obtained when ganglion-stimulating drugs were used. The responses to nicotine or DMPP varied from one preparation to another. They consisted of contraction, or relaxation, or of one followed by the other. The threshold concentrations were usually about 10^{-7} g/ml., but in some preparations DMPP did not cause a response even when the concentration was raised to 10^{-5} g/ml. Atropine (10^{-8} to 10^{-7} g/ml.) usually abolished contractions caused by nicotine or DMPP, and converted the responses to relaxations, or relaxations followed by contractions. The ganglion-blocking drugs pentolinium (10^{-5} g/ml.) and hexamethonium (5×10^{-5} g/ml.) abolished contractions and relaxations caused by either nicotine or DMPP.

Relaxations of the taenia caused by DMPP were reduced by up to 90 % by bretylium (5×10^{-6} g/ml.) and by up to 60 % by guanethidine (10^{-6} g/ml.), as shown in Fig. 9*a*. The relaxations of the taenia produced by nicotine were slightly reduced by guanethidine in a concentration of 10^{-6} g/ml. (Fig. 9*c*). These concentrations of guanethidine or bretylium were sufficient to abolish relaxations caused by stimulation of the peri-

vascular nerves (Fig. 5). Higher concentrations were needed to abolish the relaxations caused by nicotine or DMPP. The responses to nicotine or DMPP returned within 10 min of washing the guanethidine or bretylium from the bath, whereas the responses to perivascular nerve stimulation did not return, even after repeatedly washing out the bath. These differences suggest that the abolition of the responses to DMPP and nicotine caused by guanethidine and bretylium were not specifically due

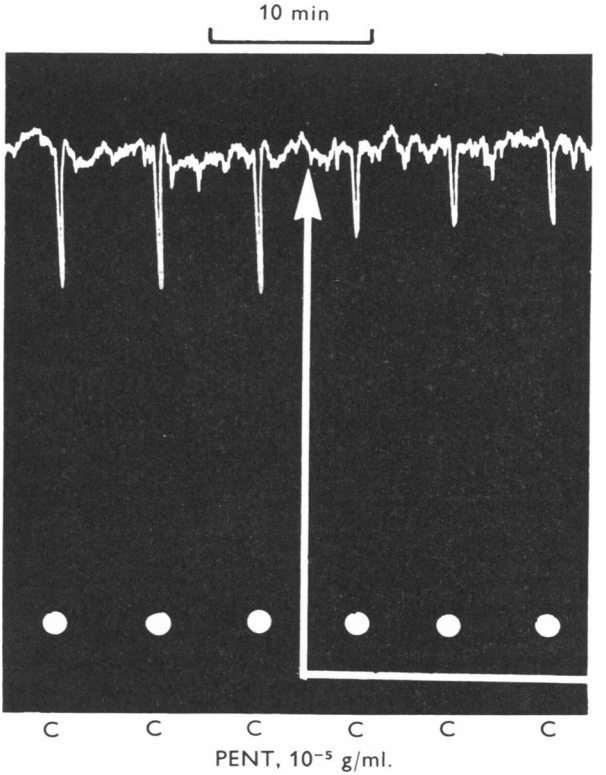

Fig. 8. Preganglionic stimulation of inhibitory neurones in the taenia. Caecal wall preparation. Pentolinium (PENT, 10^{-5} g/ml.) reduced responses of the atropinized taenia to stimulation of the flap of caecal wall with 10 pulses/sec (C, at dots). Time marker: 10 min.

to blockade of sympathetic transmission. A likely explanation for the reduction of the responses is that these drugs are exerting a ganglion-blocking action. This explanation is supported by experiments with those non-atropinized preparations in which DMPP caused contraction. Guanethidine reversed the contraction to relaxation within 10 min of its application; the excitatory response reappeared within 10 min of washing out the guanethidine (Fig. 9*b*).

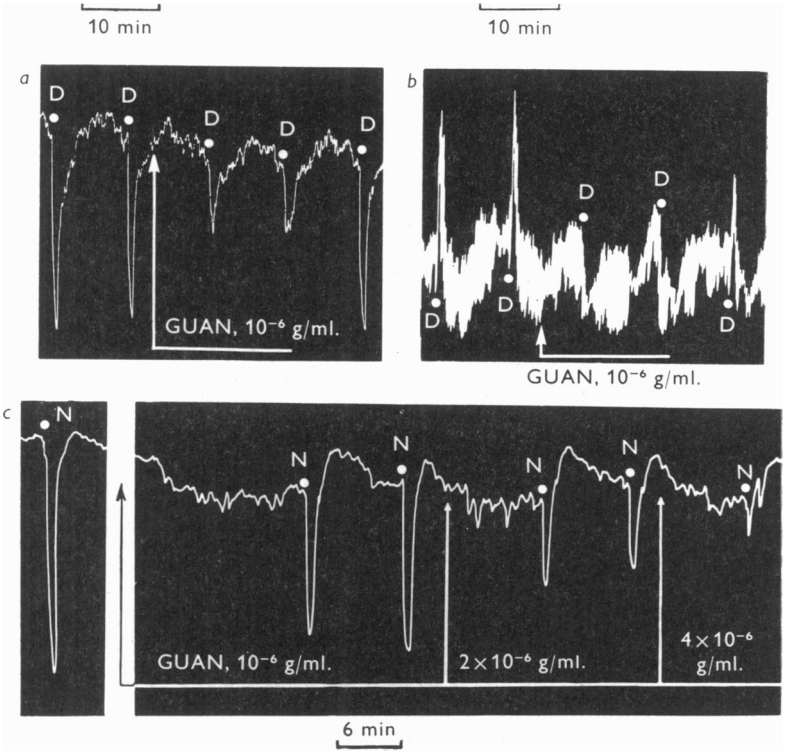

Fig. 9. Effects of ganglion-stimulating drugs on the taenia. (*a*) Taenia strip preparation treated with atropine. Guanethidine (GUAN, 10^{-6} g/ml.) reversibly reduces the inhibitory responses to DMPP (D, 5×10^{-6} g/ml., at dots, applied for 30 sec). (*b*) Taenia strip preparation, not treated with atropine. The contractions caused by DMPP (D, 5×10^{-6} g/ml., at dots, applied for 30 sec) were reversed to relaxations by guanethidine (GUAN, 10^{-6} g/ml.). Note the partial recovery of the responses within 10 min of washing out guanethidine. (*c*) Taenia strip preparation. Relaxations were produced by nicotine (N, 5×10^{-6} g/ml., at dots, applied for 1 min) although no atropine was present. These relaxations were progressively reduced by increasing concentrations of guanethidine (GUAN, 10^{-6} to 4×10^{-6} g/ml.). Time marker for (*a*) and (*b*) 10 min; and for (*c*) 6 min.

Effect of DMPP on responses to perivascular nerve stimulation. The relaxations of the atropinized taenia caused by DMPP (3×10^{-6} to 10^{-5} g/ml.), applied for periods of 15 or 30 sec at 10 min intervals, remained fairly constant in amplitude for as long as $2\frac{1}{2}$ hr. When the perivascular nerves were stimulated at intervals between applications of DMPP, the response to nerve stimulation at first increased, then decreased in amplitude over the course of about 2 hr and finally disappeared (Fig. 10). Responses to noradrenaline and to electrical stimulation of the taenia strip

were not affected by DMPP. Therefore the blockade of the perivascular nerves caused by DMPP resembles that produced by guanethidine. Wilson (1962) and Bentley (1962) observed that DMPP had a guanethidine-like action in blocking relaxations of the rabbit ileum produced by perivascular nerve stimulation.

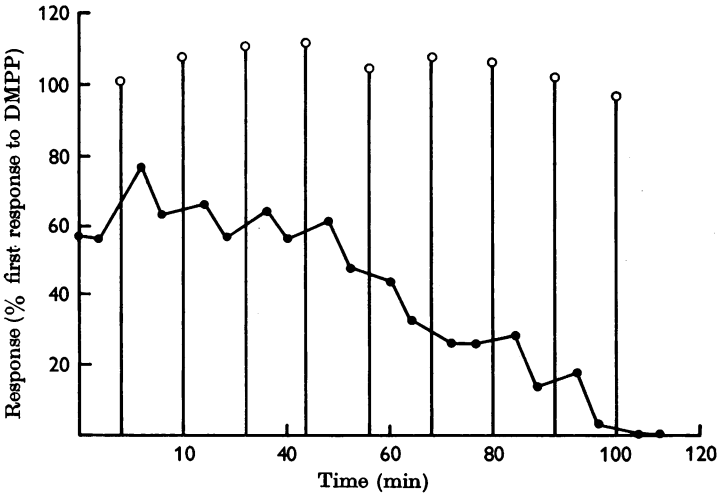

Fig. 10. Effects of DMPP on responses to perivascular nerve stimulation. The effect of repeated brief applications of DMPP in reducing responses to stimulation of the perivascular nerves. The amplitude of responses to DMPP (10^{-5} g/ml. applied for 15 sec) are shown by the open circles surmounting the vertical lines. The amplitude of responses to stimulation of the perivascular nerves with 30 pulses/sec are shown by the closed circles. Note that the responses to DMPP remained constant but that the response to stimulation of the perivascular nerves gradually decreased. Ordinate: relaxation, expressed as a percentage of the first responses to DMPP. Abscissa: time in minutes.

It is unlikely that the relaxing action of DMPP or nicotine is brought about by stimulation of sympathetic nerves, since the relaxations persisted after blocking sympathetic transmission with guanethidine, bretylium or DMPP itself. It is possible that these relaxations are caused by stimulation of another type of inhibitory neurone.

Intramural inhibitory neurones

The location of ganglion cells and axons in the guinea-pig caecum has been examined by our colleague, Dr D. C. Rogers (personal communication). The ganglion cells of Auerbach's plexus occur in clumps, lying between the taenia and the circular muscle layer towards the mid line of the taenia. Bundles of processes of the ganglion cells enter the taenia and then run parallel to its longitudinal axis. Strips of taenia, cut from the

serosal surface as in Fig. 1*b*, are practically free from ganglion cells. Therefore this preparation can be used to indicate whether DMPP acts on cell bodies or on axons.

Strips cut from the surface of the taenia from four guinea-pigs were not relaxed by DMPP (5×10^{-6} to 10^{-5} g/ml.) and some were contracted, even in the presence of atropine, as in Fig. 11*a*. These contractions can be explained by a direct action of DMPP on intestinal muscle as shown by Trendelenburg (1961). Electrical stimulation of these strips with 1–50 pulses/sec caused relaxation (Fig. 11*b*), which indicates that the axons of inhibitory neurones were still present and were still excitable.

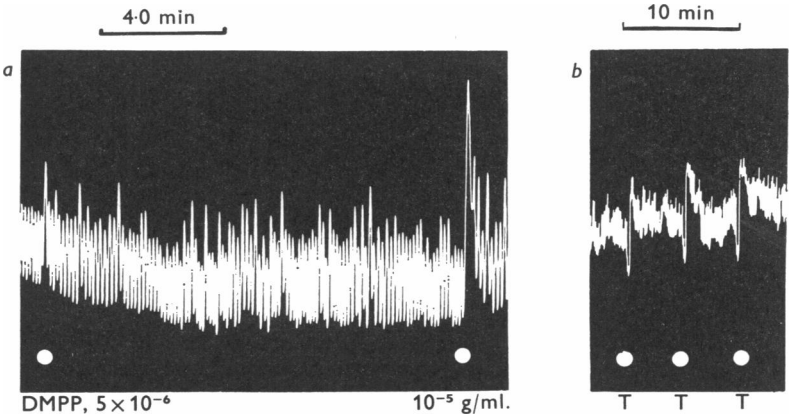

Fig. 11. Responses of ganglion-free taenia preparations. (*a*) Superficial strip of taenia. DMPP (5×10^{-6} g/ml., then 10^{-5} g/ml., at dots, applied for 30 sec) did not cause relaxation, but contracted the taenia, even in the presence of atropine (5×10^{-8} g/ml.). (*b*) Stimulation of the ganglion-free strip with 10, 20 and 30 pulses/sec (T, at dots), caused relaxation of the atropinized taenia.

The above results suggest that DMPP causes relaxation of the taenia by stimulation of ganglion cells in Auerbach's plexus and that processes of these cells innervate the taenia.

Evidence for intramural inhibitory neurones in other intestinal preparations has been obtained by Holman & Hughes (1965). They found that isolated preparations of intestine stored at 4° C lost the ability to respond to stimulation of the inhibitory perivascular nerves when they were set up again after 1–4 days, but that responses to transmural stimulation were retained. We have obtained a similar result with the taenia. The relaxations in response to stimulation across the taenia persisted after 4 days of storage at 4° C. A possible explanation for this difference is that cold storage damages the axons of the perivascular nerves, which are severed from their cell bodies, but that the intramural inhibitory neurones, which are wholly intramural and therefore intact, are resistant to damage.

5-HT. Gaddum & Picarelli (1957) suggested that 5-HT exerted its excitatory action on the guinea-pig ileum partly by stimulation of cholinergic nerves. This suggestion has been supported by many later studies. Recently Fishlock & Parks (1963) and Bucknell & Whitney (1964) showed that 5-HT caused relaxation of isolated strips of muscle from the human colon. It was of interest to determine whether 5-HT could cause relaxation of the taenia by stimulating inhibitory nerves.

In the absence of atropine, 5-HT caused contraction of taenia strips; the threshold concentration was about 10^{-9} g/ml. Atropinized preparations usually did not respond to 5-HT until a concentration of 10^{-4} g/ml. was reached. The first application of this concentration often resulted in a relaxation but subsequent applications were without effect. The extent of this tachyphylaxis was so great that the effects of drugs on the relaxation could not be tested. Some atropinized preparations were contracted by 5-HT.

DISCUSSION

Relaxations of the taenia produced by stimulation of the perivascular nerve supply are abolished by bretylium and guanethidine. The perivascular fibres are therefore similar to other sympathetic post-ganglionic nerves. However, the relaxations produced by electrical stimuli applied directly to the taenia are only reduced and not abolished by bretylium and guanethidine. Paton & Vane (1963) and Holman & Hughes (1965) have also reported that transmural stimulation of several mammalian gastrointestinal preparations causes relaxations which are not abolished by bretylium, guanethidine or xylocholine (TM 10).

Several theories regarding the origin of the relaxations which persist in the presence of adrenergic neurone-blocking drugs may be considered. First, electrical stimulation of the taenia may directly affect the smooth muscle, to cause a relaxation. Secondly, the stimuli may activate the intramuscular extensions of the perivascular nerves, but at a site which is peripheral to the point of action of bretylium and guanethidine. Thirdly, the stimuli may activate inhibitory nerves in the taenia which differ from the perivascular nerves in that they are resistant to the actions of bretylium and guanethidine.

We favour the theory that there are two different types of inhibitory nerves supplying the taenia, for the following reasons. Relaxations of the taenia, which persist in the presence of bretylium and guanethidine, can also be produced by stimulation of a flap of caecal wall attached to the taenia. Such relaxations are reduced after the application of pentolinium, a ganglion-blocking drug, but responses to stimulation of the perivascular fibres are not reduced by pentolinium. It would be possible to suggest that stimulation of the flap of caecal wall causes a direct inhibitory

response of the smooth muscle which is then conducted to the taenia, and that the conduction mechanism is sensitive to pentolinium. However, the simplest explanation of the observations is that there are preganglionic fibres in the caecal wall which make synaptic connexions with inhibitory neurones in the taenia.

Supporting evidence for the existence of such inhibitory neurones in the taenia comes from experiments with the ganglion-stimulating drugs, nicotine and DMPP. These drugs cause relaxation of the atropinized taenia. There have been several previous reports that ganglion stimulants can cause relaxations of intestinal preparations (Kuroda, 1917; Ambache, 1951; Ambache & Edwards, 1951; Levy, Michel-Ber & Cafiot, 1953; Evans & Schild, 1953; Gillespie & MacKenna, 1960; Weiss, 1962; Greeff, Kasperat & Osswald, 1962; Jarrett, 1962; Bucknell & Whitney, 1964; Burn & Gibbons, 1964). We found that the relaxing actions of nicotine and DMPP on the guinea-pig taenia are still present even though responses to sympathetic nerve stimulation had been abolished by bretylium and guanethidine. Similarly, Gillespie & MacKenna (1960) found that nicotine produced relaxations of the rabbit colon when the effects of perivascular nerve stimulation were abolished by xylocholine. These observations suggest that the relaxations caused by nicotine and DMPP are mediated by a structure which is not affected by bretylium and guanethidine. In addition we found that superficial strips of the taenia, which do not contain nerve cell bodies, are not relaxed by DMPP. This implies that the relaxations caused by ganglion stimulants are due to excitation of inhibitory neurones with their cell bodies in Auerbach's plexus. Ambache (1951) and Ambache & Edwards (1951) suggested that the inhibitory effects of nicotine on rabbit and cat intestine were due to excitation of intramural inhibitory ganglion cells. It is reasonable to suppose that the cell bodies of the intramural inhibitory neurones in the taenia that are stimulated by nicotine and DMPP are identical with those that are innervated by preganglionic fibres from the caecal wall, and that electrical stimulation of the taenia excites axons of the same neurones.

It is apparent that the intramural inhibitory neurones of the guinea-pig taenia differ in some way from the perivascular sympathetic nerves, but we are uncertain about the transmitter substance released from them. It is possible that they are adrenergic, but attempts to demonstrate (or disprove) this by depletion of catecholamines with reserpine, blockade of α- or β-adrenoreceptors with appropriate blocking drugs or enhancement of responses to released catecholamines with cocaine, have given equivocal results (Burnstock, Campbell & Rand, unpublished observations).

We have shown that inhibitory responses of the taenia to nicotine and DMPP still occur when the responses to sympathetic nerve stimulation

are abolished by bretylium or guanethidine. However, it should be pointed out that other workers have found that inhibitory responses of gut preparations to nicotinic stimulating drugs were abolished by bretylium (Greeff *et al.* 1962; Jarrett, 1962; Burn & Gibbons, 1964). We also observed that, when the concentrations of guanethidine or bretylium were increased to about five times those necessary to abolish responses to sympathetic stimulation, the inhibitory responses of the taenia to nicotine and DMPP were abolished. However, these concentrations also abolished the excitatory responses of the taenia (in the absence of atropine) to nicotine or DMPP. Jarrett (1962) made a similar observation on the rabbit ileocolic sphincter. The abolition of both the inhibitory and the excitatory responses of the taenia to nicotine and DMPP is readily reversible by washing the bretylium or guanethidine out of the bath, in contrast to the almost irreversible abolition of responses to perivascular nerve stimulation. These observations can be explained by the ganglion-blocking action of bretylium and guanethidine, which has been reported previously (Kosterlitz & Lees, 1961; Gokhale, Gulati, Kelkar & Joshi, 1963).

It should also be noted that Evans & Schild (1953) found that a plexus-free preparation of the circular muscle from cat jejunum was relaxed by nicotine, whereas the ganglion-free strip of taenia was not relaxed by DMPP. It appears that, in the cat jejunum, the action of nicotine was not exerted on intramural neurones, but on nerve endings. Nicotine and other drugs with a similar effect also produce responses in many tissues in which the effect does not appear to be due to stimulation of intrinsic ganglion cells. For instance, nicotine increases the force of contraction of papillary muscle (Lee & Shideman, 1959), and causes pilo-erection in the cat's tail (Coon & Rothman, 1940), vasoconstriction in the rabbit's ear (Kottegoda, 1953), contraction of the cat nictitating membrane (Thompson, 1958), and contraction of the spleen capsule of the dog (Daly & Scott, 1961) and cat (Brandon & Rand, 1961). After section and degeneration of the sympathetic supply to the pilo-erector muscles, rabbit ear and cat nictitating membrane the actions of nicotine are reduced or abolished (Burn, Leach, Rand & Thompson, 1959), and it is likely that the site of action of nicotine in these tissues is the sympathetic nerve endings. Also, Gillespie & MacKenna (1960) reported that sympathetic denervation of the rabbit colon resulted in loss of the inhibitory response to nicotine. Yet we have noted above that Gillespie & MacKenna found that the inhibitory response to nicotine was not abolished by xylocholine. We can offer no explanation for this apparent paradox.

The role of the intramural inhibitory neurones in the caecum may be to produce relaxation of the taenia and thus allow the caecum to fill. The guinea-pig caecum does not exhibit peristaltic activity (Elliott & Barclay-

Smith, 1904), but it is possible that intramural inhibitory nerves in the small intestine mediate the descending inhibition which occurs during peristalsis (Bayliss & Starling, 1899). Hukuhara, Yamagami & Nakayama (1958) have elicited descending inhibition in the intestine by chemical and mechanical stimulation of the mucosa and shown that this inhibition is abolished by hexamethonium.

It must be asked whether the intramural inhibitory nerves receive connexions from the central nervous system. Langley (1922) suggested that the vagi innervate both excitatory and inhibitory neurones in the gastro-intestinal tract. Ambache (1951) and Ambache & Edwards (1951) agree with this interpretation. Many workers have shown that there are inhibitory fibres to the gastro-intestinal tract in the vagus nerve (Langley, 1898; Bayliss & Starling, 1899; McSwiney & Robson, 1929; Greeff *et al.* 1962; Martinson & Muren, 1963; Paton & Vane, 1963). In fact, Auer & Meltzer (1907) reported that the movements of the rabbit caecum were arrested by vagus nerve stimulation. However, Greeff *et al.* (1962) and Paton & Vane (1963) have shown that the vagal inhibition of the stomach is blocked by bretylium and xylocholine, respectively. The vagal inhibitory fibres are preganglionic (Bayliss & Starling, 1899; Greeff *et al.* 1962; Paton & Vane, 1963). Therefore the blockade caused by bretylium and xylocholine may be a result of their ganglion blocking actions.

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